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### *Transplantation of schistosome sporocysts between host snails:*

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## Transplantation of *schistosome* sporocysts between host snails: A video guide [version 1; referees: 2 approved]

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## Abstract



Schistosomiasis is an important parasitic disease, touching roughly 200 million people worldwide. The causative agents are different *Schistosoma* species. Schistosomes have a complex life cycle, with a freshwater snail as intermediate host. After infection, sporocysts develop inside the snail host and give rise to human dwelling larvae. We present here a detailed step-by-step video instruction in English, French, Spanish and Portuguese that shows how these sporocysts can be manipulated and transferred from one snail to another. This procedure provides a technical basis for different types of *ex vivo* modifications, such as those used in functional genomics studies.

## Keywords

Schistosomiasis, sporocyst transfer, *Biomphalaria*, video instruction

## Open Peer Review

Referee Status:  

	Invited Referees	
	1	2
version 1		
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1 Patrick C. Hanington , University of  
Alberta, Canada

**2 Christopher J. Bayne**, Oregon State University, USA

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**Author roles:** **Mouahid G:** Conceptualization, Methodology, Resources, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Rognon A:** Methodology, Resources, Visualization, Writing – Review & Editing; **de Carvalho Augusto R:** Visualization, Writing – Review & Editing; **Driguez P:** Methodology, Visualization, Writing – Review & Editing; **Geyer K:** Methodology, Visualization, Writing – Review & Editing; **Karinshak S:** Methodology, Visualization, Writing – Review & Editing; **Luviano N:** Visualization, Writing – Review & Editing; **Mann V:** Methodology, Visualization, Writing – Review & Editing; **Quack T:** Methodology, Visualization, Writing – Review & Editing; **Rawlinson K:** Methodology, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Wendt G:** Methodology, Visualization, Writing – Review & Editing; **Grunau C:** Funding Acquisition, Methodology, Project Administration, Supervision, Visualization, Writing – Review & Editing; **Moné H:** Conceptualization, Methodology, Project Administration, Resources, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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## Introduction

Schistosomiasis is an acute and chronic parasitic disease caused by blood fluke trematodes of the genus *Schistosoma*. Estimates show that at least 206.5 million people required preventive treatment in 2016 (WHO, 2017). This disease is spread throughout tropical and subtropical areas of Africa, South America, Middle-East and Asia and, more recently, it represents an emerging risk in Europe (Holtfreter *et al.*, 2014).

The schistosome lifecycle includes two obligatory hosts (Figure 1a):

- (I) A freshwater snail, in which stages of the parasite, called sporocysts, multiply asexually and produce the mammalian infecting larvae, known as the cercariae, and
- (II) A mammalian host in which the parasites (males and females) develop to sexual maturity, pair, mate and produce eggs.

Eggs are excreted from the mammalian host in faeces or urine, and if they reach bodies of freshwater a second free-swimming larval stage, called the miracidium, hatches and infects the snail by active penetration through the integument. After penetration, the miracidium loses the ciliated plates, and develops in less than 18 hours into a mother sporocyst. Daughter sporocysts develop inside the mother sporocyst and eventually escape to invade the snail tissues and organs, mainly the ovotestis and the hepatopancreas. In each daughter sporocyst, germinal cells (similar to stem cells) divide and differentiate to produce either cercariae (cercariogenous sporocysts), or further daughter sporocysts (sporocystogenous sporocysts).

The asexual multiplication of the daughter sporocysts continues throughout the time that the parasite remains resident in the

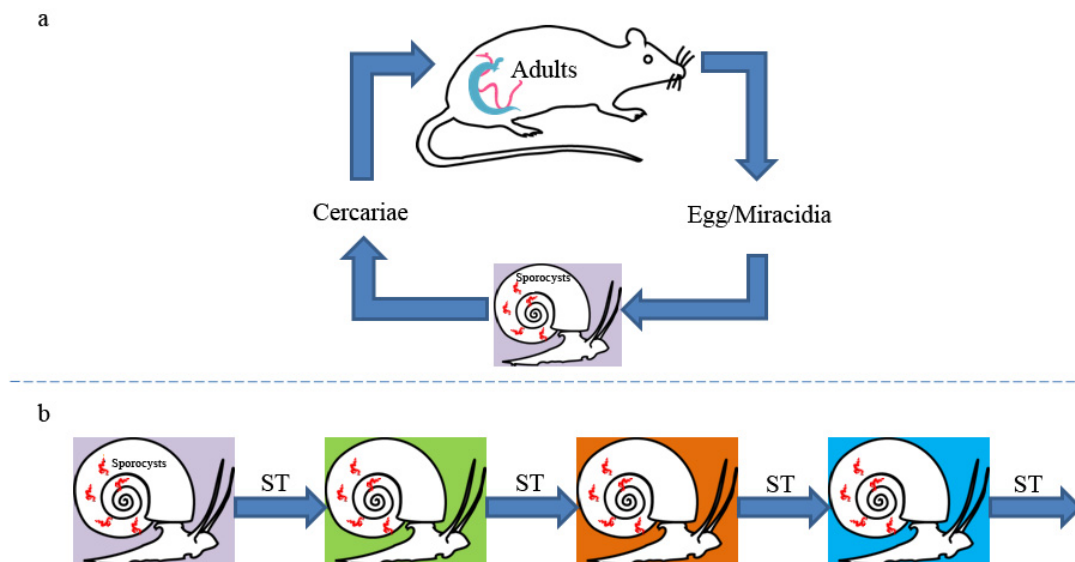
snail (Théron, 1981). Several generations of daughter sporocysts may occur in the same individual snail, because daughter sporocysts can undergo a re-differentiation into new daughter sporocysts, whether they were sporocystogenous or cercariogenous (Combes *et al.*, 1983). We exploited this reversible developmental plasticity to design a method, called “sporocyst transfer” or “transplantation”, generating a direct lifecycle (snail to snail transmission) and bypassing the mammalian host and free-swimming larval stages (Jourdane *et al.*, 1984) (Figure 1b). This technique, first developed by Chernin (1966) for *Schistosoma mansoni*, consists of transplanting schistosome sporocysts from one donor snail to recipient snails. It was improved in our laboratory for *S. mansoni* with the snail *Biomphalaria glabrata* as host (Jourdane, 1977; Jourdane & Théron, 1980; Jourdane & Combes, 1989). We also adapted the procedure to other species of schistosomes like *S. haematobium* and the snail *Planorbium metidjensis* (Kéchemir & Combes, 1982), and *S. bovis* in *Bulinus truncatus* (Jourdane *et al.*, 1984). We describe here, in a video documentary, a detailed and improved method that enables the generation of clonal populations of schistosomes.

The technique of microsurgical transplantation requires a precise protocol and specific skills in snail handling and surgery that can be quite difficult to establish in a laboratory. Therefore, the aim of this publication is to provide for the scientific community a technical video in order to make this technique more broadly available.

## Methods

### Design and development of the video on the transplantation technique

The video explains all the steps, equipment, reagents and manipulations necessary for the technique to be applied by researchers in their own laboratories. It is provided in French



**Figure 1.** (a) The canonical lifecycle of schistosomes includes relatively long periods of development in the two obligatory hosts (a mammal and a snail) and two short-lived, free-swimming larval stages. (b) The reduced lifecycle of schistosomes is exclusive to the snail host, thanks to the technique of sporocyst transfer or transplantation, and bypasses the mammalian host and the free-swimming larval stages. ST: Sporocysts Transfer.

(Video 1), English (Video 2), Spanish (Video 3) and Portuguese (Video 4). There are seven obligatory steps (see protocol in Supplementary File 1). The first step explains the preparation of the donor snail, using an antibiotic solution. The second step explains how to prepare the recipient snails, using an anesthetic solution. The third step provides information on how to prepare the work space and which equipment is needed. The fourth step details the dissection of the donor snail. The fifth step explains how to isolate the sporocyst grafts. The sixth step shows the different manipulations necessary to conduct the transplantation technique properly. The seventh and last step explains how to maintain the recipient snails after the transplantation. The manufacturing process of the two specific tools necessary for the sporocysts transfer, i.e. the microretractor and the glass microsyringe, is presented in Video 5 and Supplementary File 2. Housing, feeding and animal care followed the national ethical standards established in the writ of February 1st, 2013 (NOR: AGRG1238753A). The French Ministère de l'Agriculture et de la Pêche and the French Ministère de l'Education Nationale de la Recherche et de la Technologie provided permit A66040 to the laboratory for animal experiments and certificate to the experimenters (authorization 007083, decree 87–848). All efforts were made to ameliorate any suffering of animals. For details see Supplementary File 1 and the video on sporocyst transfer.

## Discussion

The technical video was designed to make the technique of transplantation of schistosome sporocysts widely available so that it can be used by researchers from different scientific backgrounds, with different levels of research experience and from different countries, including those that speak English, French, Spanish and Portuguese. Many applications of microsurgical transplantation of sporocysts have been highlighted previously (Jourdan & Combes, 1989) such as:

- (i) Selection and maintenance of “pure” and stabilised strains with high compatibility to snails (cloning avoids genetic recombination), resistance to certain antihelminthics, or with specific chronotypes,
- (ii) Avoidance of genetic drift (for example in *S. haematobium* where there is a loss of compatibility towards the snail after 2 or 3 passages in the mammal),
- (iii) Compensation for the very low success rate of monomiracidial infections required to produce single sexes of the parasite,
- (iv) Maintenance of male and female clones without being forced to identify the sex,
- (v) Constitution of genomic libraries,
- (vi) Improving the ability to conduct schistosome genetic-based studies, or
- (vii) Studying mollusc immunology.

Additional uses of the sporocyst transplantation technique are envisioned based on recent molecular breakthroughs afforded by RNA interference gene knockdown- (Alrefaei *et al.*, 2011; Mann *et al.*, 2010) and Clustered Regularly Interspaced Short

Palindromic Repeats (CRISPR-Cas9) gene knockout- (Jinek *et al.*, 2012) strategies. Use of the sporocyst transplantation technique in combination with these targeted manipulation strategies will assist the scientific community in functional genomics-based studies of schistosome biology directly in the snail host.

The conditions for successful transplantation are the following: proper anesthesia of the recipient snails; obtaining highest prevalence and post-transplantation survival of the recipient snails; ability of the daughter sporocyst grafts to produce new generation of daughter sporocysts; ability of the new generation of daughter sporocysts to produce enough cercariae (or sporocysts); ability of these cercariae to infect the mammal definitive host (infectivity) and produce eggs with viable and infective miracidia; and the ability to successfully perform several consecutive transplantations.

## Data availability

**Video 1:** Technique de transplantation microchirurgicale de sporocystes de schistosomes.

<http://doi.org/10.5281/zenodo.1116997> (Mouahid *et al.*, 2017a)

**Video 2:** Technique of microsurgical transplantation of schistosomes sporocysts.

<http://doi.org/10.5281/zenodo.1117007> (Mouahid *et al.*, 2017b)

**Video 3:** Técnica de trasplatación microquirúrgica de esporocistos de *Schistosoma*.

<http://doi.org/10.5281/zenodo.1117009> (Mouahid *et al.*, 2017c)

**Video 4:** Técnica microcirúrgica de transplante de esporocistos de esquistosomas.

<http://doi.org/10.5281/zenodo.1117011> (Mouahid *et al.*, 2017d)

**Video 5:** Manufacture of tools for microsurgical transplantation of schistosome sporocysts.

<http://doi.org/10.5281/zenodo.1117017> (Mouahid *et al.*, 2017e)

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## Competing interests

No competing interests were disclosed.

## Grant information

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*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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We would like to thank Joris Fabryka from Platinum UPVD for his help in the production of this video. The IHPE is a WHO Collaborating Center on the Hosts-Schistosomes Interactions (FRA-69).

## Supplementary material

Supplementary File 1: Step-by-step protocol of the technique of microsurgical transplantation of schistosome sporocysts.

[Click here to access the data.](#)

Supplementary File 2: Instructions for the manufacture of the tools for sporocyst transfer by microsurgical transplantation.

[Click here to access the data.](#)

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# Open Peer Review

Current Referee Status:



Version 1

Referee Report 07 February 2018

doi:10.21956/wellcomeopenres.14646.r29641



**Christopher J. Bayne**

Department of Integrative Biology, College of Science, Oregon State University, Corvallis, OR, USA

Mouahid *et al* provide a useful description of sporocyst transplantation. The utility of the protocol is clearly stated and well illustrated in the videos. The comments below are offered as means to improve the precision of the text, and its comprehension.

## TEXT

There is no statement as to the success rates achieved in this complex protocol. It would be helpful if, at a minimum, readers were assured that infective cercariae have been routinely obtained from transplanted schistosomes.

The Legend to Figure 1 can be made more clear by rewording as follows:

“(b) The cycle can be artificially simplified in a process that avoids the definitive mammalian host: sporocysts are transferred or transplanted between snails, bypassing the mammalian host and the free-swimming larval stages. ST: Sporocyst Transfer.”

Also, the text below Figure 1 (small font) appears to be lacking one or more initial words. Please check this. Perhaps “Sporocyst transplantation between...”

the ciliated plates, and, **if the penetrated snail is a susceptible individual,** develops in less

hepathopancreas = hepatopancreas

cercariae (**in** cercariogenous sporocysts), or further daughter sporocysts (**in** sporocystogenous sporocysts).

schistosomes like *S. haematobium* = schistosomes **including** *S. haematobium*

laboratories. It is provided in French... = laboratpries. Commentaries are provided in French...

produce single sexes of the parasite = produce single sex **populations** of the parasite

forced to identify the sex, = forced to identify the sex (only the adults are sexually dimorphic),

gene knockout- ([Jinek et al., 2012](#)) strategies. *Delete hyphen.*



produce new generation = produce a new generation

## VIDEO

The high quality of the video is noted.

It is appreciated that modification of the commentary may be, at this stage, challenging. The only version that I have checked is the English one. It is noted that the phrases accompanying the relaxation protocol include this: "Five hours later the snails are asleep, released and ready to..." "released" should be "relaxed". This is not an essential change.

I have some concern over the use of PBS. Is this mammalian PBS? Is there a formulation (that can be included in the publication) for a PBS that is physiological for pulmonate snails and their sporocysts? Perhaps the authors use such a formulation. If so, this needs to be clear as some others may assume that mammalian PBS is suitable. And, in the big picture, the techniques work, so a snail PBS may not be essential. But the success rate may be improved if a non-physiological PBS is replaced by a physiological one. The osmotic concentrations of *Biomphalaria* and mammals are markedly different, as described by another of Eli Chernin's publications<sup>1</sup>.

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**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Molluscan schistosomiasis, including in vitro methodologies.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 24 January 2018

doi:[10.21956/wellcomeopenres.14646.r29642](https://doi.org/10.21956/wellcomeopenres.14646.r29642)





**Patrick C. Hanington** 

School of Public Health, University of Alberta, Edmonton, AB, Canada

This is an excellent, step-by-step account of the schistosome sporocyst transplantation method developed and refined by this group. The accompanying video and written methodological summary clearly outline each stage of the procedure. Videos are also provided to assist with the development of specific tools to undertake the presented method and generate all required materials.

The brief written introduction and discussion accurately touch on the importance of this technique and its utility and integration with many tools intended to manipulate the snail/schistosome interface, such as RNAi.

A fantastic resource for those working in this system. That the videos are made available in multiple languages ensures that many of those working in with these model organisms will be able to access this important resource.

In the future, it would be great to see accompanying videos explaining any alternations of the technique that are required for implementation of this technique in *Bulinus* sp. snails.

**Is the rationale for developing the new method (or application) clearly explained?**

Yes

**Is the description of the method technically sound?**

Yes

**Are sufficient details provided to allow replication of the method development and its use by others?**

Yes

**If any results are presented, are all the source data underlying the results available to ensure full reproducibility?**

No source data required

**Are the conclusions about the method and its performance adequately supported by the findings presented in the article?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**